

1 **Supplementary information for**

2 Title: Ecological restoration alters microbial communities in mine tailings profiles

3 Author names: Yang Li¹, Zhongjun Jia², Qingye Sun^{1,*}, Jing Zhan¹, Yang Yang¹, Dan
4 Wang¹

5 ¹School of Resources and Environmental Engineering, Anhui University,
6 Hefei, Anhui Province, China

7 ²State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil
8 Science, Chinese Academy of Sciences, Nanjing, Jiangsu Province, China

9 *Correspondence should be addressed to Qingye Sun at

10 E-mail: sunqingye@ahu.edu.cn; Tel/Fax: +86-551-6386-1882

11 **This file includes:**

12 1. Supplementary Table S1-S3

13 2. Supplementary Figure S1-S9

14

15 **Supplementary Tables**

16 Supplementary Table S1 F values of pairwise comparisons of Bray-Curtis similarity
17 index among different plant covered of profiles by one-way non-parametric
18 multivariate analysis of variance (NPMANOVA) analysis. Single asterisks values
19 indicated significant difference at the level < 0.05 and double asterisks values
20 indicated significant difference at the level < 0.01; n.s. indicated non-significant
21 differences; non-significant differences was found among different plant species in
22 horizons 20-60cm (data not shown).

horizon	BW	VZ	IC
0-10cm	BW	16.787 **	14.179 **
	VZ		n.s.
	IC		
10-20cm	BW	8.233 **	11.490 **
	VZ		n.s.
	IC		

23

24

25 Supplementary Table S2 The Pearson correlation(r) between main identified bacterial
 26 taxonomic groups, i.e. phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and
 27 classes *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*
 28 and *Deltaproteobacteria*, (within *Proteobacteria* phylum) and environment
 29 parameters. Single asterisk show the significant difference at the level < 0.05, and
 30 double asterisk show the significant difference at the level < 0.01.

	LOI	TN	pH	Water con.	As	Cu	Fe	Pb	Zn
<i>Acidobacteria</i>	0.384**	0.286*	0.247	-0.03	-0.507**	0.164	0.181	0.120	0.424**
<i>Actinobacteria</i>	0.361**	0.449**	-0.189	0.225	-0.166	0.126	0.548**	0.431**	-0.068
<i>Bacteroidetes</i>	-0.212	-0.391**	0.434**	-0.256	-0.069	0.156	-0.397**	0.188	0.109
<i>Firmicutes</i>	-0.025	0.207	-0.647**	0.396**	0.260	-0.231	0.496**	-0.038	-0.561**
<i>Nitrospira</i>	-0.166	0.103	-0.567**	0.122	0.442**	-0.114	0.125	-0.294*	-0.298*
<i>Alphaproteobacteria</i>	0.632**	0.531**	0.049	0.049	-0.496**	0.168	0.359**	0.101	0.389**
<i>Betaproteobacteria</i>	-0.252	-0.295*	0.164	-0.195	0.156	-0.078	-0.537**	-0.308*	0.106
<i>Gammaproteobacteria</i>	-0.263	-0.131	-0.294*	0.065	0.461**	-0.278*	-0.106	-0.374**	-0.284*
<i>Deltaproteobacteria</i>	0.280*	0.145	0.322*	-0.251	-0.206	0.145	-0.033	-0.087	0.419**

31

32

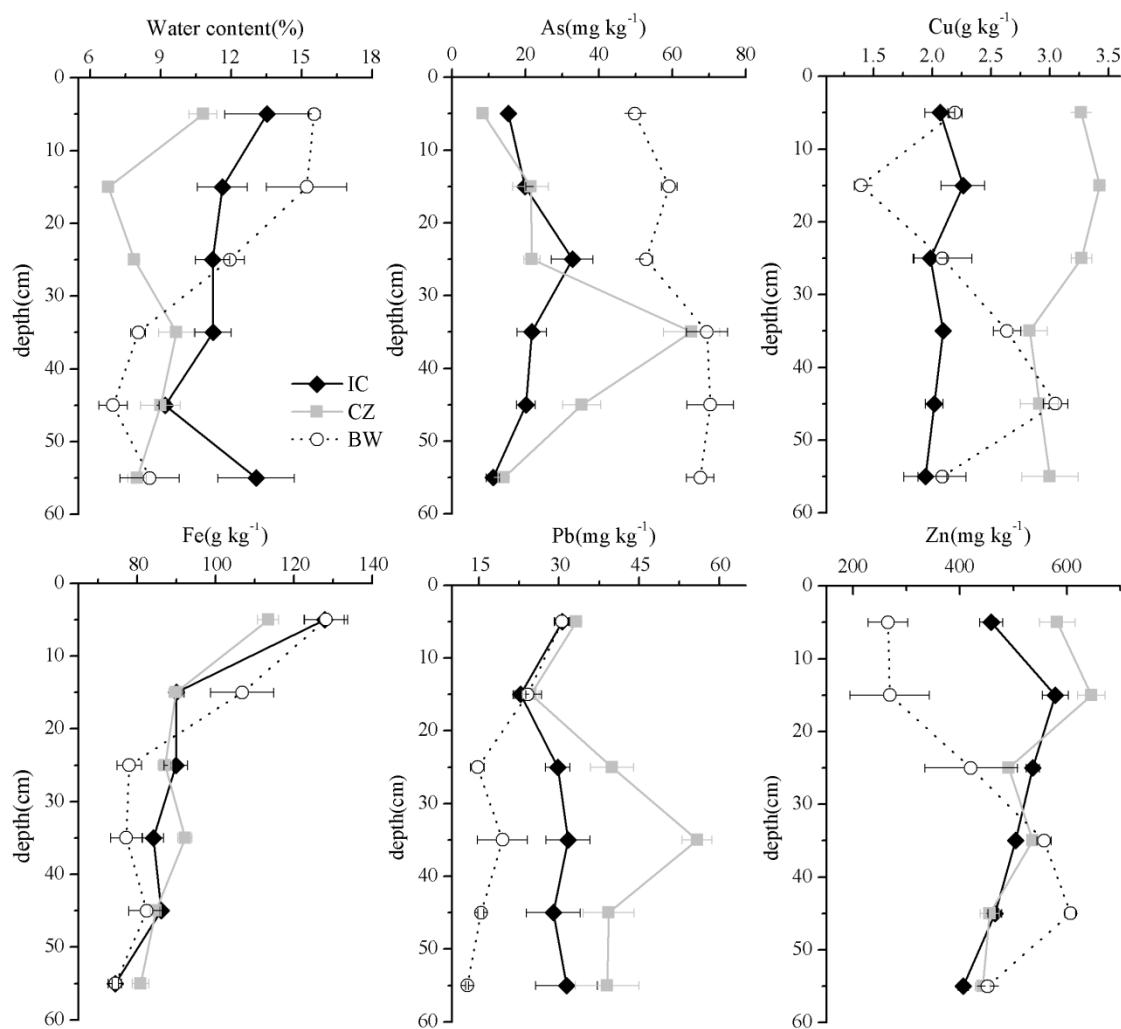
33 Supplementary Table S3 Diversity and predicted richness of *nifH* sequences from the
34 clone libraries*

	clones number	OTUs number	Coverage (%)	Shannon index (H')	Simpson's index	Pielou evenness index
BW(DNA)	108	12	100	2.13	0.85	0.45
IC(DNA)	108	27	97.2	3.03	0.94	0.65
VZ(DNA)	107	27	94.4	3.05	0.94	0.65
IC(cDNA)	106	16	98.1	2.29	0.86	0.49
VZ(cDNA)	108	17	100	2.57	0.90	0.55

35 *OTUs were defined by a 3% difference in the nucleic acid sequence alignment for the
36 *nifH* gene.

37

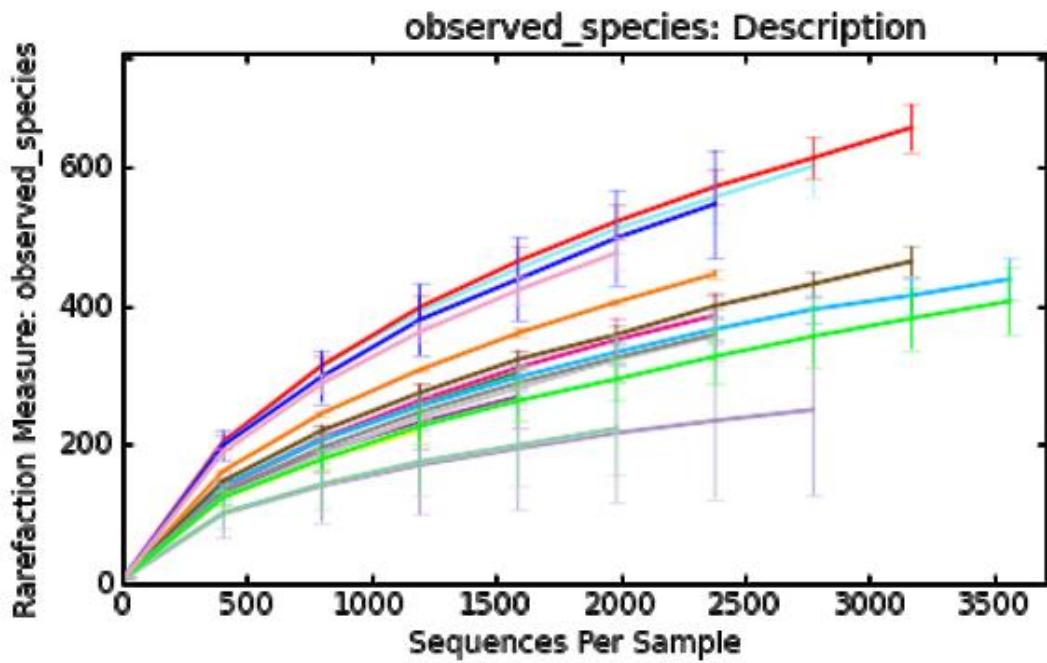
38 **Supplementary figures:**



39

40 Supplementary Fig.S1 Physico-chemical properties of 6 horizons from different plant
 41 types of profiles. The error bars showed the standard error of relative abundance of
 42 the three subsamples for each tailings sample. IC and CZ: tailings revegetated by
 43 *Imperata cylindrica* and *Chrysopogon zizanioides*, BW: bare wasteland.

44

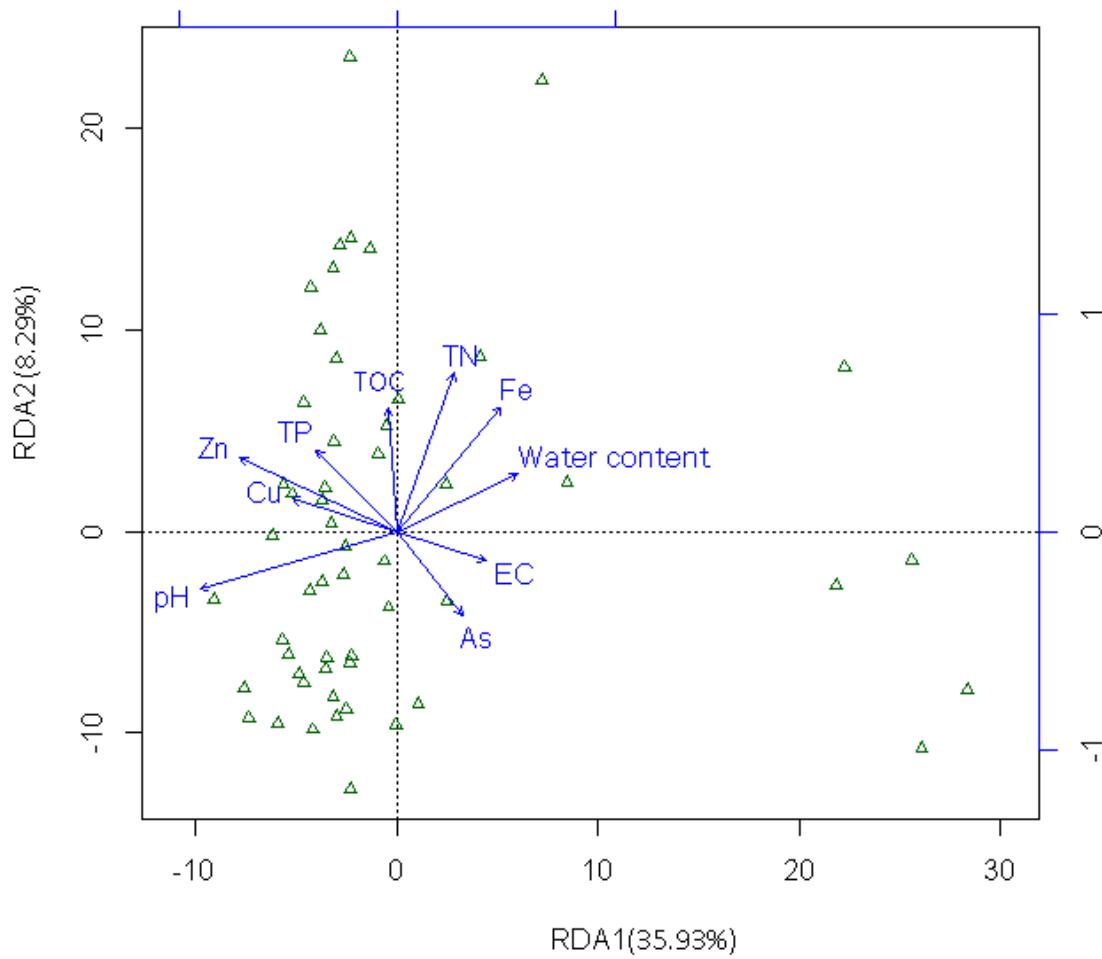


45

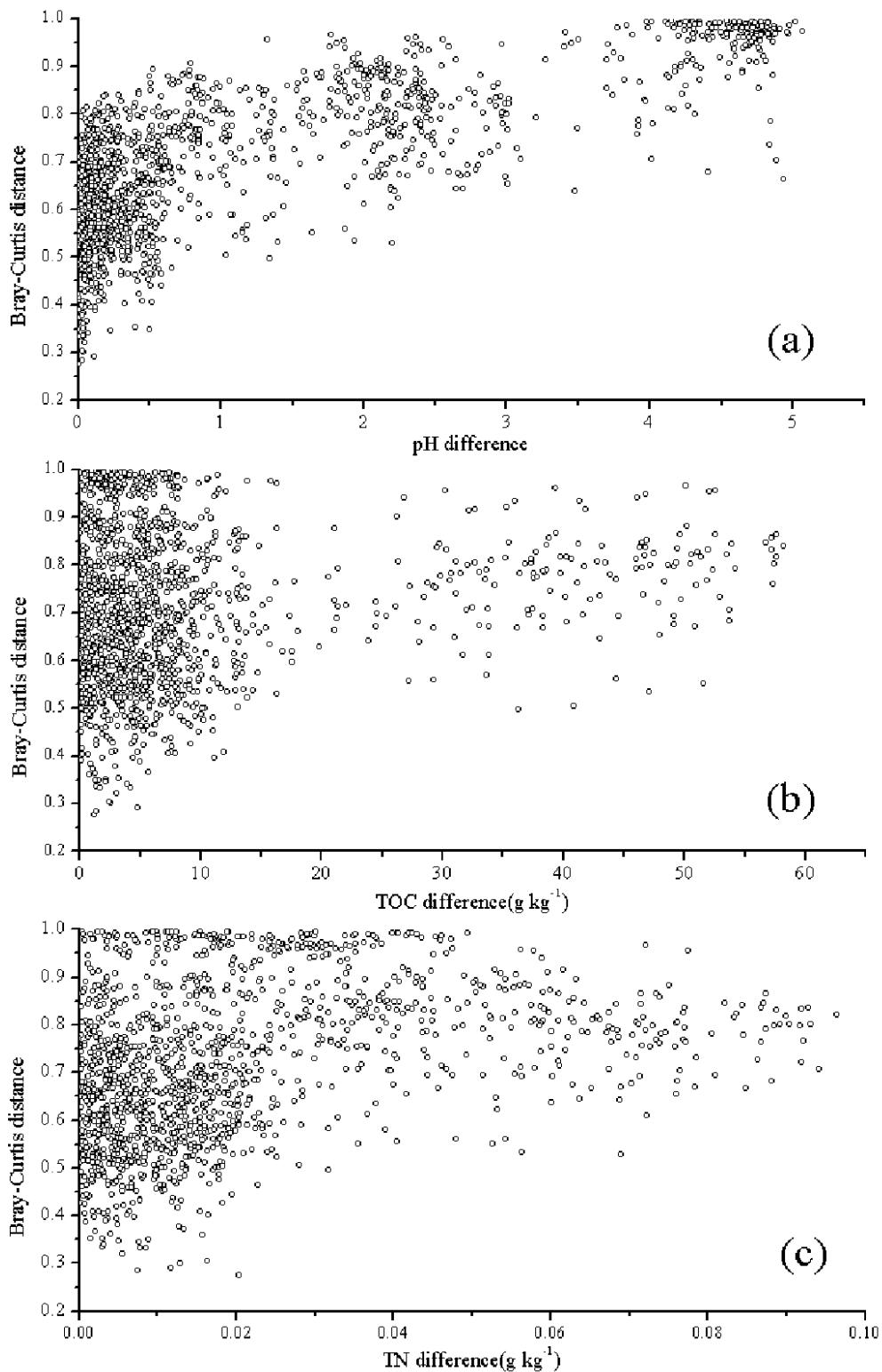
46

47 Supplementary Fig. S2 Rarefaction curve of OTUs (observed_species) recovered from
48 mine tailings.

49



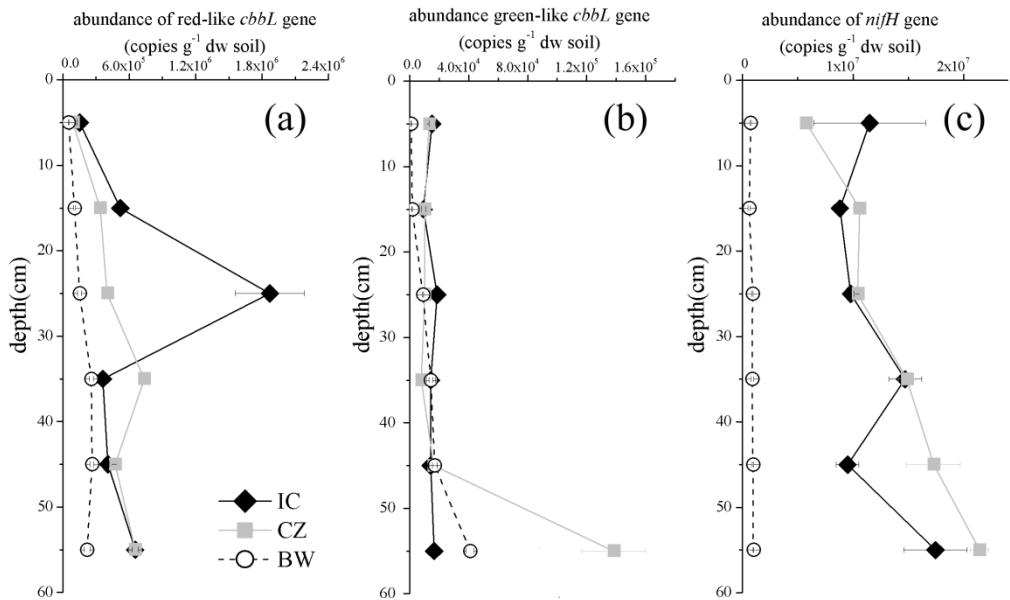
50
 51 Supplementary Fig.S3 Redundancy analysis (RDA) for 6 horizons of different plant
 52 species with forward selection of predictor variables followed by Monte Carlo
 53 permutations (999 permutations). Solid arrows represent predictor (chemical)
 54 variables significantly associated ($P<0.05$) with the variation in the bacterial
 55 community structure.
 56



57

58 Supplementary Fig. S4 Relationships between (a) pH, (b) TOC and (c) TN and
 59 bacterial community diversity (Bray-Curtis similarity on the basis of phylotype
 60 composition).

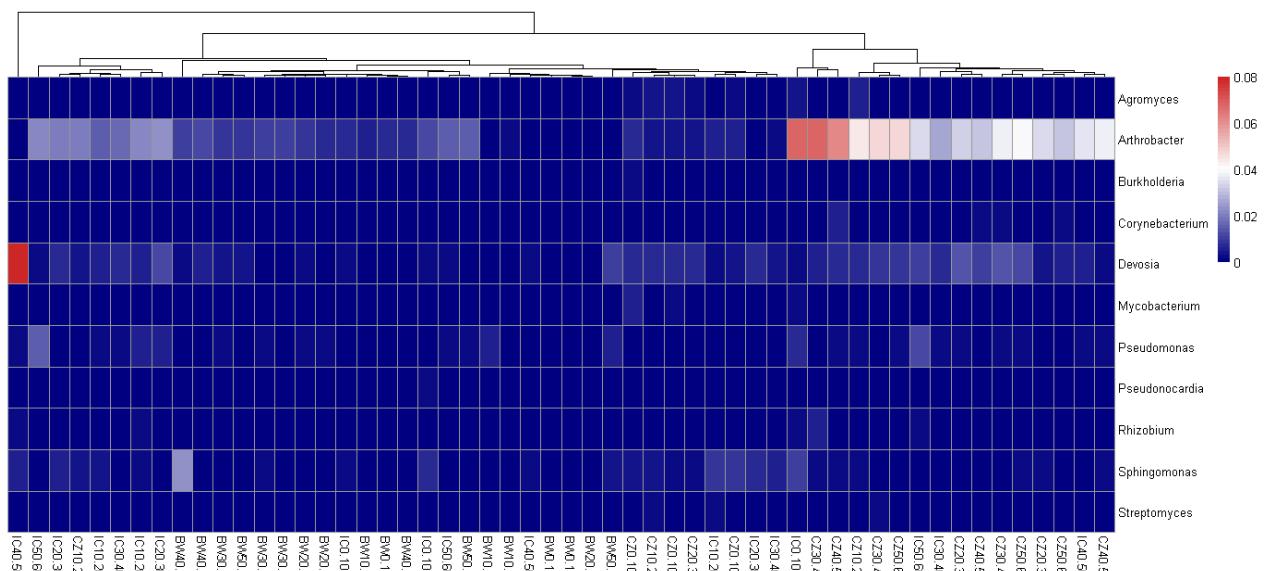
61



62

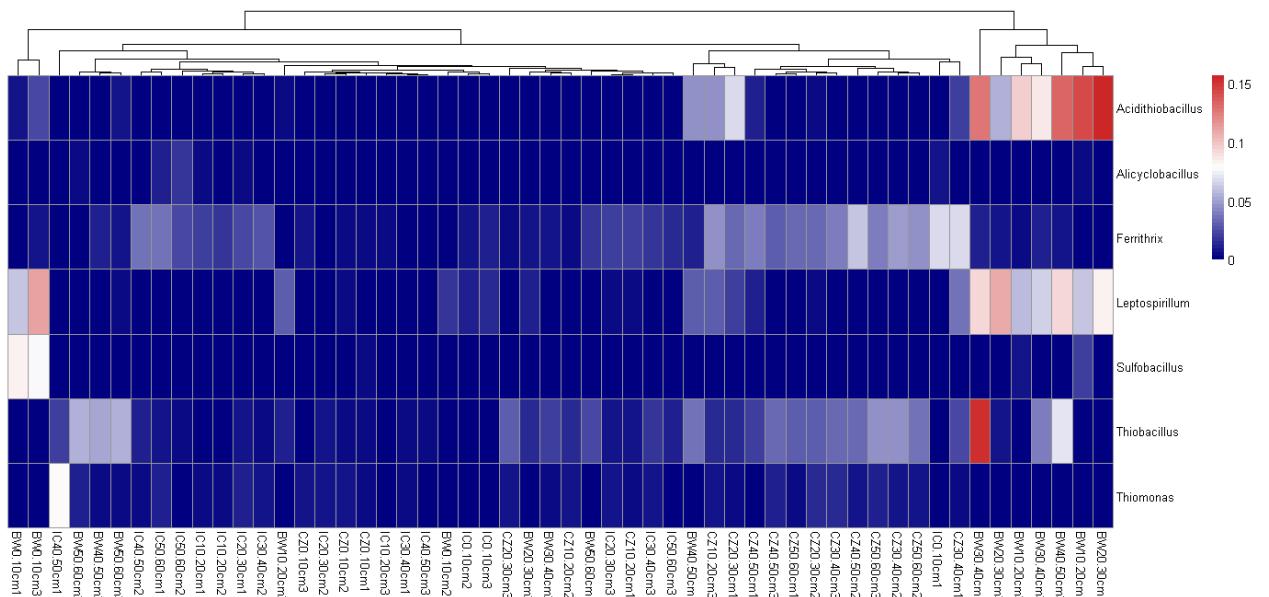
63 Supplementary Fig.S5 Abundance of *cbbL* (red-like(a) and green-like(b)) and *nifH* (c)
64 genes along the profiles (copies g⁻¹dry soil, n=3). Error bars indicated standard error.
65 IC and CZ: tailings revegetated by *I. cylindrica* and *C. zizanioides*, BW: bare
66 wasteland. The *cbbL* gene encoded the large subunit of form I ribulose
67 1,5-bisphosphate carboxylase/oxygenase (RubisCO) which was the first and
68 rate-limiting step in Calvin-Benson-basham (CBB) reductive pentose phosphate
69 pathway, in which form I RubisCO can be divided into two major groups, green-like
70 and red-like, in terms of the amino acid compositions and length of branches in the
71 phylogenetic tree. And *nifH* gene encoded dinitrogenase reductase which is a key
72 enzyme of nitrogen fixation.

73 (a)



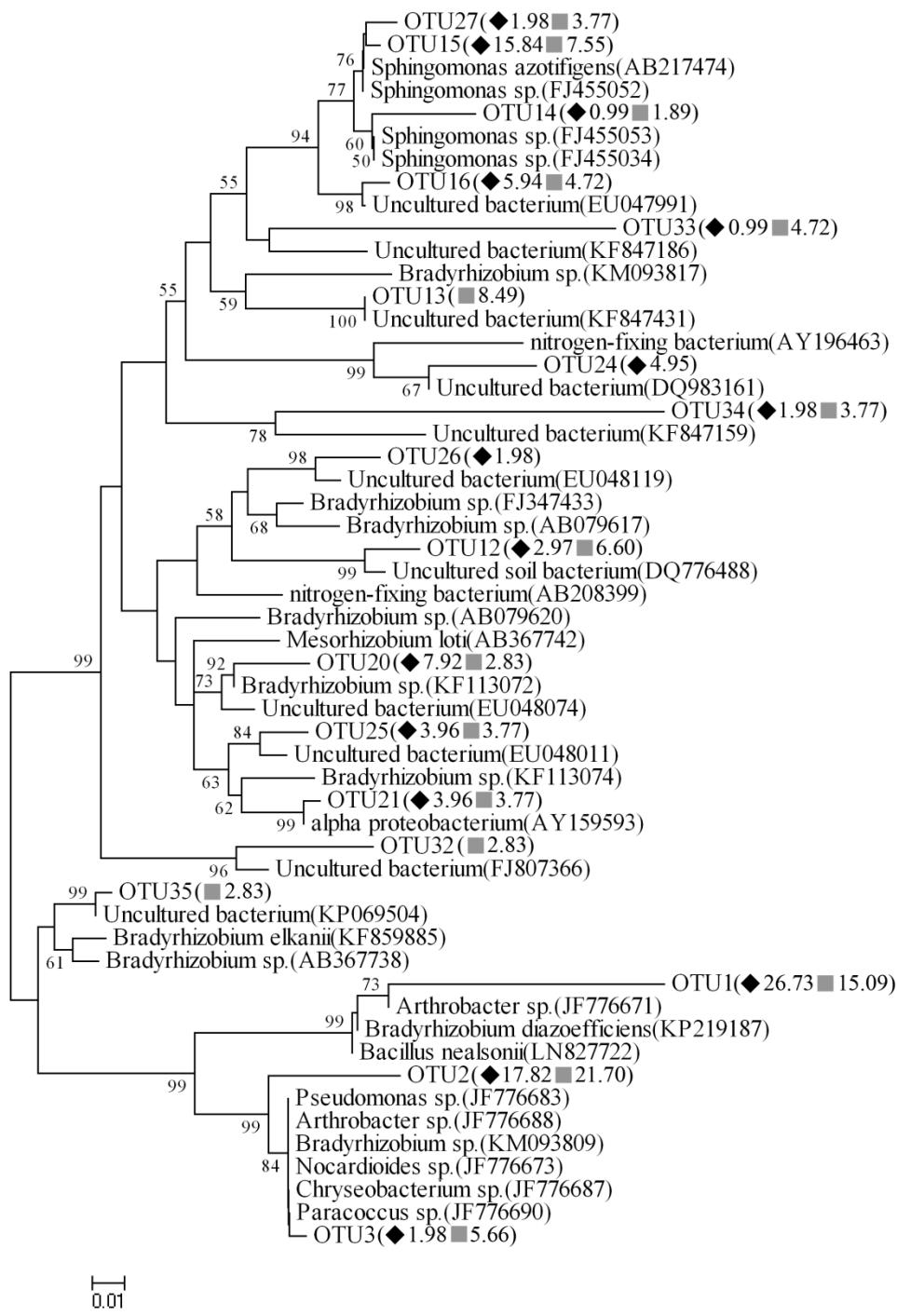
74

75 (b)

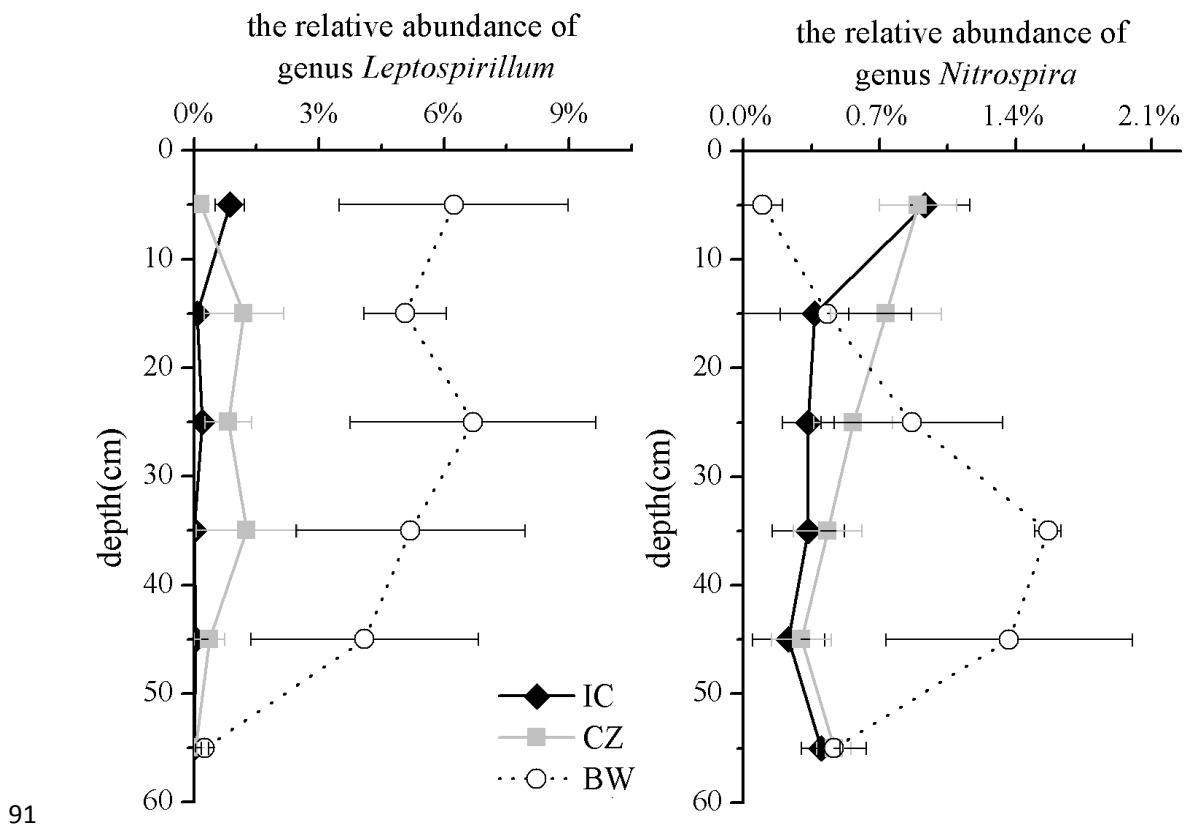


76

77 Supplementary Fig.S6 Heat map for nitrogen- fixing genera(a), and iron- & sulfur-
 78 oxidizing bacteria (b) in 6 horizons of different plant species. The genera of
 79 *Agromyces*^{1, 2}, *Arthrobacter*¹⁻⁴, *Burkholderia*⁵, *Corynebacterium*⁶, *Devosia*⁷,
 80 *Mycobacterium*^{4, 8}, *Pseudomonas*⁹, *Pseudonocardia*², *Rhizobium*⁹, *Sphingomonas*¹⁰
 81 and *Streptomyces*⁴ were likely to be involved in nitrogen fixation. 7 genera
 82 (*Acidithiobacillus*¹¹, *Alicyclobacillus*¹², *Ferrithrix*¹³, *Leptospirillum*¹¹, *Sulfobacillus*¹²,
 83 ¹⁴, *Thiobacillus*¹¹ and *Thiomonas*¹⁵) related with iron- and sulfur- oxidizing were
 84 found in this study.



85 0.01
86 Supplementary Fig.S7 Phylogenetic tree of transcriptional *nifH* sequences in
87 rhizosphere. Clones from the present study are marked with rhizosphere of *I.*
88 *cylindrica* (IC) and rhizosphere of *C. zizanioides* (CZ). Additional symbols show the
89 relative frequency (%) of a sequence in their respective clone libraries (◆, IC; ■, CZ).
90 Bootstrap values of >50% are exhibited at branch points.



91
92 Supplementary Fig.S8 Relative abundance (percentage) of genera of *Leptospirillum*
93 and *Nitrospira* both of which belonging to phylum *Nitrospira*. The error bars showed
94 the standard error of relative abundance of the three subsamples for each tailings
95 sample. IC and CZ: tailings revegetated by *I. cylindrica* and *C. zizanioides*, BW: bare
96 wasteland.
97



98

99 Supplementary Fig. S9 Examples of the colonizer plant species in the Shuimuchong
100 tailings pond. BW: bare wasteland; IC and CZ: tailings revegetated by *I. cylindrica*
101 and *C. zizanioides*.

102

103

104 **References**

- 105 1. Sellstedt, A. & Richau, K.H. Aspects of nitrogen-fixing *Actinobacteria*, in particular
106 free-living and symbiotic Frankia. *FEMS Microbiol. Lett.* **342**, 179-186 (2013).
- 107 2. Gtari, M., Ghodhbane-Gtari, F., Nouiou, I., Beauchemin, N. & Tisa, L.S. Phylogenetic
108 perspectives of nitrogen-fixing actinobacteria. *Arch. Microbiol.* **194**, 3-11 (2012).
- 109 3. Smyk, B. Fixation of atmospheric nitrogen by the strains of *Arthrobacter*. *Zentralblatt für
110 Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Zweite
111 naturwissenschaftliche Abt.: Allgemeine, landwirtschaftliche und technische Mikrobiologie*
112 **124**, 231 (1970).
- 113 4. Young, J. Phylogenetic classification of nitrogen-fixing organisms. *Biological nitrogen
114 fixation*, 43-86 (1992).
- 115 5. Chen, W.-M. et al. Legume symbiotic nitrogen fixation by β-proteobacteria is widespread in
116 nature. *J. Bacteriol.* **185**, 7266-7272 (2003).
- 117 6. Berndt, H., Lowe, D.J. & Yates, M.G. The Nitrogen-Fixing System of *Corynebacterium
118 autotrophicum*. *Eur. J. Biochem.* **86**, 133-142 (1978).
- 119 7. Rivas, R. et al. Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in
120 symbiosis with *Neptunia natans*, an aquatic legume from India. *Syst. Appl. Microbiol.* **26**,
121 47-53 (2003).
- 122 8. Biggins, D. & Postgate, J. Nitrogen fixation by cultures and cell-free extracts of
123 *Mycobacterium flavum* 301. *Microbiology* **56**, 181-193 (1969).
- 124 9. Derylo, M. & Skorupska, A. Enhancement of symbiotic nitrogen fixation by vitamin-secreting
125 fluorescent *Pseudomonas*. *Plant Soil* **154**, 211-217 (1993).
- 126 10. Videira, S.S., De Araujo, J.L.S., Rodrigues, L.D.S., Baldani, V.L.D. & Baldani, J.I.
127 Occurrence and diversity of nitrogen-fixing *Sphingomonas* bacteria associated with rice plants
128 grown in Brazil. *FEMS Microbiol. Lett.* **293**, 11-19 (2009).
- 129 11. Dold, B. Evolution of Acid Mine Drainage formation in sulphidic mine tailings. *Minerals* **4**,
130 621-641 (2014).
- 131 12. Karavaiko, G.I. et al. Reclassification of ‘*Sulfobacillus thermosulfidooxidans*’ subsp.
132 *thermotolerans*’ strain K1 as *Alicyclobacillus tolerans* sp. nov. and *Sulfobacillus*
133 *disulfidooxidans* Dufresne et al. 1996 as *Alicyclobacillus disulfidooxidans* comb. nov., and
134 emended description of the genus *Alicyclobacillus*. *Int. J. Syst. Evol. Micr.* **55**, 941-947
135 (2005).
- 136 13. Johnson, D.B., Bacelar-Nicolau, P., Okibe, N., Thomas, A. & Hallberg, K.B. *Ferrimicrobium
137 acidiphilum* gen. nov., sp. nov. and *Ferrithrix thermotolerans* gen. nov., sp. nov.: heterotrophic,
138 iron-oxidizing, extremely acidophilic actinobacteria. *Int. J. Syst. Evol. Micr.* **59**, 1082-1089
139 (2009).
- 140 14. Stott, M., Watling, H., Franzmann, P. & Sutton, D. The role of iron-hydroxy precipitates in the
141 passivation of chalcopyrite during bioleaching. *Miner. Eng.* **13**, 1117-1127 (2000).
- 142 15. Coupland, K. et al. Oxidation of iron, sulfur and arsenic in mine waters and mine wastes: an
143 important role for novel *Thiomonas* spp. (2004).